Conservation genetics in the new molecular age

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Molecular techniques can be used to address questions of conservation significance. At the individual level, these questions concern how kinship affects reproduction, group structure, dispersal, and cooperation which leads to social group assembly rules such that populations can be genetically managed and restored. Furthermore, inbreeding can now be measured at the individual level in natural populations, and, in combination with field studies, can be used to assess fitness declines that might require active management to arrest. We discuss genetic units for conservation and attempt to integrate data on phenotype and the environment into an evaluation that includes genetic data. To a limited extent, genetic surveys can now include genes that may influence fitness, as well as those not under selection. We discuss the use of animal and plant remains to monitor current populations and to determine directly the demographic changes that have occurred in the past.

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Conservation genetics has entered a new age, in which a tremendous array of genomic resources can be used to categorize diversity at multiple evolutionary levels, from the kinship of individuals to relationships of populations and species. Current assessments of genetic diversity are based largely on neutral variation (genetic variation that does not affect fitness) and provide essential information about population history and demographics. The measurement of genetic variation in fitness-related genes has been an intractable problem in conservation genetics, yet the underlying genetic variation that influences the short and long-term survival of populations is likely to depend on it.

Now, we are able to examine variation in genes influencing fitness as well. Moreover, conservation genetics studies are incorporating new sources of information such as isotope profiles (Clegg *et al.* 2003), geographic information system (GIS) and landscape databases (Ji Wei and Leberg 2002; Hoffmann *et al.* 2003; Manel *et al.* 2003), adaptive traits (Smith *et al.* 1997; Blondel *et al.* 1999; McKay *et al.* 2001), pathogenic organisms (Sehgal *et al.* 2001), and behavioral and ecological information (Ross 2001). Conservation assessments can now involve the integration of genetic, ecological, and phenotypic information, to maximize the likelihood that populations will persist given future challenges, as well as preserving the historical legacy of populations (Crandall *et al.* 2000; Moritz 2002).

The application of molecular techniques to conservation questions has recently been well explored (Frankham *et al.* 2002). Consequently, here we will focus on a subset of ecological and conservation questions that may now be resolved by the advent of new molecular genetic techniques and methods of analysis. We begin by discussing standard molecular techniques and methods, and how they have been used to address two primary themes of conservation genetics over the past few decades. This is followed by a selection of current and future applications of molecular techniques to conservation questions, ranging from the individual (relatedness, inbreeding, and fitness) to the population level (gene flow, migration, and units for conservation). We also discuss the use of non-invasive typing of plant and animal remains, both recent and ancient, to address these questions. We illustrate these applications with examples from conservation genetics studies of carnivores (Wayne 1996; Wayne *et al.* in press), but the techniques and questions are widely applicable to the study of plants and animals (Table 1).

In a nutshell:

- Molecular techniques have been used to monitor the genetic variability of declining populations and to assess their evolutionary uniqueness
- Today, a wide range of conservation issues can be addressed, including kinship, social structure, inbreeding depression, migration rate, and population assignment
- New non-invasive approaches allow the DNA typing of trace remains
- A current goal of conservation genetics is to directly assess variation in fitness-related genes and those influenced primarily by genetic drift

The past

For decades, the field of conservation genetics was focused on two questions. The first concerned levels of genetic variation in populations, and the risk that low

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Table 1. The appropriate uses of molecular techniques and the difficulty and costs associated with genetic typing

"No" indicates the technique is not appropriate due to technical limitations or lack of statistical power; "limited" indicates the technique may be of some use but is limited by statistical assumptions and power; "yes" indicates the technique is appropriate. See Moritz and Mable (1996) for a discussion of molecular techniques.

Techniques	Karyology[1]	Protein electrophoresis[2]	mtDNA sequencing[3]	Nuclear gene sequencing[4]	Mini-satellite[5]	Micro-satellite[6]	RAPD, AFLP[7]
Relatedness, paternity, inbreeding	No	Limited	Limited	Limited	Yes	Yes	Yes
Genealogical hierarchies, units for conservation	No	Limited	Yes	Limited	Limited	Yes	Limited
Population assignment, gene flow, migration	No	Limited	Limited	Limited	Limited	Yes	Limited
Adaptation	No	Limited	Limited	Yes	No	Limited	Limited
Non-invasive demographic monitoring	No	No	Yes	Limited	No	Yes	Limited
Historic and ancient DNA	No	No	Yes	Limited	No	Limited	No
Difficulty	Moderate	Moderate	Low	Low	Moderate	Moderate	Moderate
Cost	Low	Low	Low	Low	Low	Moderate	Moderate

[1]A technique to visualize chromosomes providing information on chromosome number and morphology. [2]Involves the separation of protein variants from a single gene in an electric field in a porous medium. [3]Involves DNA sequencing of mitochondrial genes and DNA segments. [4]Involves DNA sequencing of nuclear genes including exons, introns, and non-transcribed regions. [5]Involves the separation of minisatellite repeats producing a multilocus genetic fingerprint. [6]Involves the separation of microsatellite repeats producing a single locus pattern (see Figure 1). [7]Randomly Amplified Polymorphic Fragments and Amplified Fragment-Length Polymorphisms, two techniques for quantifying variation in random genomic segments.

levels of variation and future genetic losses posed to their continued existence. A typical study might estimate variation across the range of a species for several loci thought to be largely unaffected by selection, and hence indicative of variation in the genomic background. Populations with low levels of variation were presumed to be most at risk of the immediate problem of inbreeding depression (reduction in fitness due to inbreeding), and of future losses of variation that are critical for an effective adaptive response to changing conditions.

A second question focused on the degree of historical isolation and the level of gene flow (genetically effective migration) between populations. Here, genetic analysis provided an assessment of the potential for populations to exchange migrants, and identified genetically divergent populations. If a population has been isolated for a very long time, it might be considered an evolutionarily significant unit (ESU). ESUs have the potential to become new species and may be adaptively divergent (Moritz 1994). Because they are distinct, ESUs warrant separate management and conservation and, in the US, they might fall under the protection of the Endangered Species Act. Consequently, the identification of distinct populations has become an important management and policy objective.

The majority of conservation genetic studies have relied on mitochondrial DNA, chloroplast DNA, and nuclear microsatellite loci (Figure 1), all of which are generally assumed to be selectively neutral and highly variable genetic markers, suitable for population-level analyses. The choice of marker depends on the type of question being asked, the relative ease of application to the species of interest, and cost, among other concerns (Table 1).

The present and future

Relatedness, paternity, inbreeding, and fitness

Levels of genomic variation and population distinction are critical issues. However, this focus of study has obscured other genetic issues of great importance to population management and to the evolutionary processes that will enable populations to survive. One general problem is that past genetic analyses have viewed populations as a whole – freely breeding units – and have probed little into the genealogical structure within populations. In part, this tendency reflected a lack of genetic markers that were variable enough to estimate relatedness accurately. However, the kinship structure within populations may be instrumental in their survival over the short and long term. In vertebrates, kin-based social structure is common, and the presence of kin may increase reproductive success or cause reproductive suppression if close relatives are present. Furthermore, individuals may tend to disperse to areas with few close relatives (see below). Consequently, knowing the detailed genealogical structure of a population and how it influences behavior and demography is essential for conservation planning.

Recent genetic studies using microsatellite markers have shown how kinship affects reproduction, group structure, cooperation, and dispersal (Ross 2001). For example, in the African wild dogs (*Lycaon pictus*) of Kruger National Park,

South Africa, nine packs were studied by telemetry methods for nearly a decade (Figure 2). Researchers monitored dispersal patterns and the social dynamics within packs. A genetic analysis using microsatellite loci was able to assign individuals to their packs of origin correctly in, on average, 75% of attempts and determined the reproductive success of each adult and the genetic relationship of individuals within and between packs (Girman *et al.* 1997). The relatedness data showed that packs were formed by same-sex siblings (Figure 2) from which a single breeding pair was recruited. Offspring of the pair from previous years, as well as all adult siblings, cooperated in the feeding of young, food acquisition, and pack defense. However, if one of the breeding pair was replaced by a non-relative, the siblings of the replaced individual soon migrated to other areas where they had a high proportion of close relatives. Such rules of social group assembly, dissolution, and interaction should guide reintroduction and genetic augmentation programs, and should be used to improve the breeding success of endangered species in captivity.

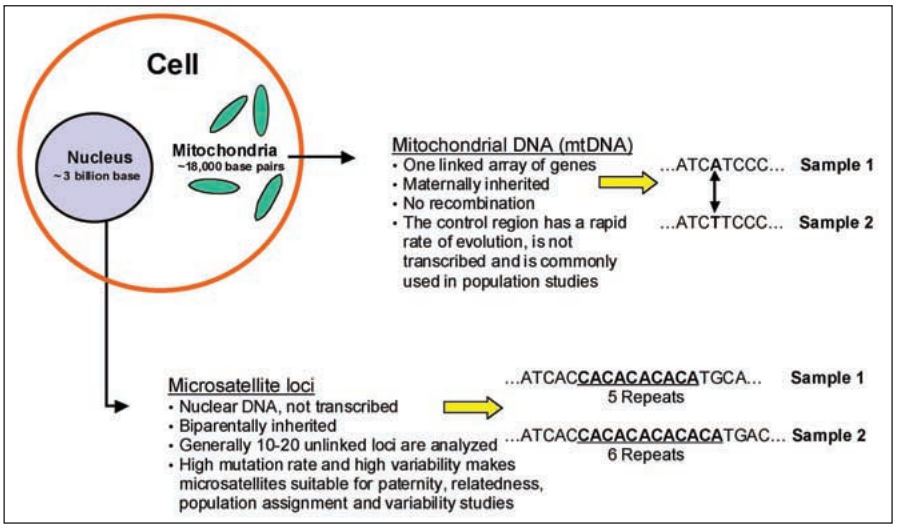

Figure 1. *The two dominant marker systems in conservation genetics. Mitochondrial DNA sequence data (top) is obtained by amplification of DNA from genes or DNA segments within the mitochondrial genome. The double-headed arrow points to a nucleotide site that differs between the two sequences (a nucleotide substitution). Microsatellite loci (below) consist of a variable number of short repeats of 2–5 nucleotides. The alleles of a microsatellite locus differ by the number of repeat units.*

Breeding among close relatives leads to a reduction in genetic variability and increases the likelihood that genes having a deleterious effect on fitness will be expressed. Both of these genetic changes can result in a reduction in fitness and could conceivably cause a population decline. In the wild, inbreeding depression has been shown to affect birth weight, survival, reproduction, resistance to disease, predation, and responses to environmental stress and global warming (Keller and Waller 2002; Schiegg *et al.* 2002). To detect inbreeding depression, the genealogical relatedness of individuals needs to be compared to estimates of fitness (Amos and Balmford 2001). For example, Amos *et al.* (2001) assessed parental relatedness in the long-finned pilot whale (*Globicephala melas*), the grey seal (*Halichoerus grypus*), and three species of albatross (*Diomedea exulaus*, *Thalassarche chrysostoma*, and *Thalassarche melanophris*). In these species, there was a significant negative relationship between parental similarity and reproductive success. This was apparent even at moderate levels of relatedness, suggesting that parents more dissimilar than average derive fitness benefits. In the same way, it was recently shown that grey seals from genetically similar parents had a greater frequency of illness (Acevedo-Whitehouse *et al.* 2003).

Genealogical hierarchies and units for conservation

Molecular techniques are commonly used to reconstruct the genealogical associations of populations (Avise 2000). For example, in the endangered island fox (*Urocyon littoralis*) on California's Channel Islands, six island subspecies are defined by a genealogical analysis (Figure 3). These subspecies are arranged in a hierarchy, placing northern and southern populations in different groups and identifying populations that are most closely related. The fox relationship tree implies that the San Nicholas and San Clemente Island populations are divergent (Wayne *et al.* 1991; Goldstein *et al.* 1999), whereas the San Miguel and Santa Rosa Island populations are more closely related and are the last populations to have been isolated. Recently, the San Miguel Island population has gone extinct in the wild due to predation by non-native golden eagles (Roemer *et al.* 2002).

The genealogical analysis suggests that the Santa Rosa Island foxes might be considered as a genetic source to augment the captive breeding population of San Miguel Island foxes, or as a source for reintroduction should the captive breeding program fail. In contrast, because of their levels of distinctiveness, northern and southern island populations should not be mixed. Consequently, evolutionary hierarchies can help order conservation priorities by identifying populations that have been distinct for a long time and providing guidance for genetic management, both in the wild and in captivity.

Population assignment, gene flow, and migration

Recently, analytical techniques have been developed to assign individuals to specific populations based on microsatellite data, and to assess the contribution to the genome of each individual from different source populations (Pritchard *et al.* 2000; Blanchong *et al.* 2002; Manel *et al.* 2002). This is useful information for several conser-

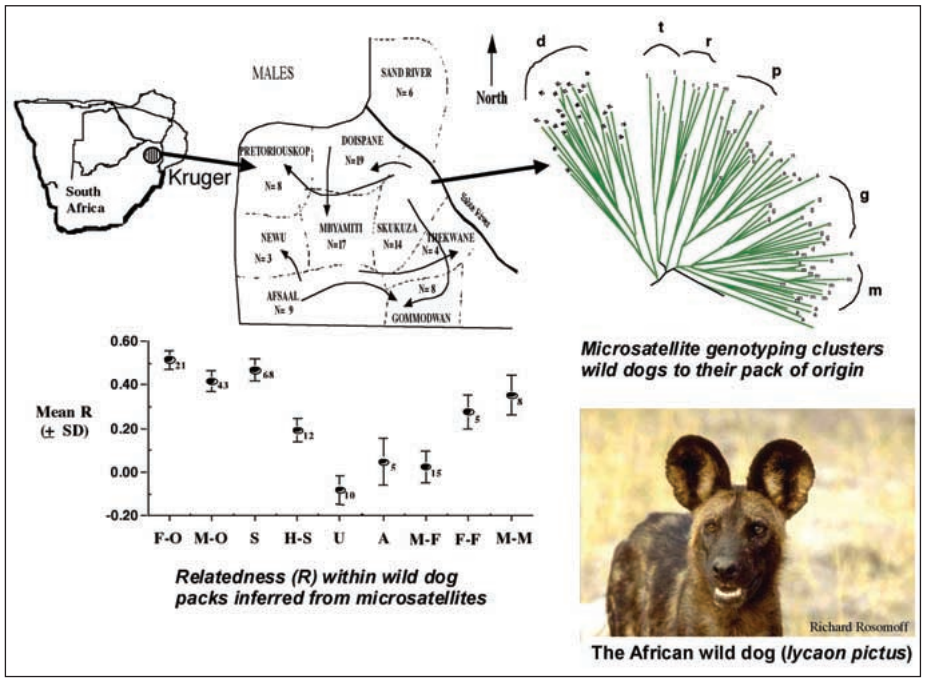

Figure 2. *Location of nine wild dog packs in Kruger National Park, South Africa (left) and observed dispersal events caused by a change in pack relatedness hierarchy. Relationship tree of individuals as indicated by lines with letters from the nine wild dog packs (top right). Pack clusters are indicated with the first letter of the pack name. Relatedness of individuals in different social groupings is shown at lower left. These relatedness values demonstrate that female parent and offspring (F–O), male parent and offspring (M–O) and siblings (S) have the expected relatedness values of 0.5 (sharing 50% of genes), whereas unrelated individuals (U) and the alpha breeding pair (A) are unrelated (r = 0). Same-sex male (M–M) and female (F–F) non-breeding adults have high relatedness consistent with their being half-sibling (H–F), cousins, or siblings (Girman et al. 1997). In contrast, opposite sexed adults (M–F) appear unrelated (r near zero).*

vation problems. For example, of 202 foxes studied for 17 microsatellite loci, only two were not assigned to their population of origin (Figure 3). This suggests that foxes of unknown origin, or biological material derived from them, can be used to deduce the source population (Blanchong *et al.* 2002; Manel *et al.* 2002). Using a similar approach, population, herd, and social group boundaries may be defined, and trace samples can be matched to individual populations. For example, samples of whale, turtle meat, and caviar sold in markets can be traced to their population of origin to determine if they were legally obtained (Birstein *et al.* 1998; Palumbi and Cipriano 1998).

One critical distinction for conservation is the potential difference between gene flow and migration. The former involves the exchange of individuals between populations who succeed in reproducing, and often refers to a historical process occurring over many generations. In contrast, migration may only involve the recent movement of individuals between populations, some of whom may reproduce. Population assignment data can be used to estimate migration rates if we assume that an individual assigned to a population different from that in which it is found is actually a migrant from the assigned population (Blanchong *et al.* 2002; Manel *et al.* 2002). For example, the two foxes that were misassigned to Santa Cruz Island could be recent migrants to Santa Rosa Island (Figure 3). The origin of colonists can also be identified. For example, microsatellite data were used to determine which of the nine possible island populations served as a source for gray seals (*H grypus*) that had recently colonized three of the Orkney Islands (Gaggiotti *et al.* 2002). The source populations were not readily predicted based on distance alone. The estimation of migration rates is critical information for metapopulation models used to predict future demographic changes, and determining the origins of colonists allows us to identify the sources of recruits most likely to rescue declining populations. Furthermore, migration rates can be compared in disturbed and undisturbed populations to assess the effects of habitat fragmentation (Stow *et al.* 2001).

Non-invasive monitoring

An exciting new possibility for monitoring the demography of species that are difficult to observe and capture utilizes organic material that organisms leave behind. New molecular techniques allow the extraction of DNA from animal and plant remains such as feces, feathers, hair, bone, and fish scales, revealing a non-invasive genetic record of individuals (Morin and Woodruff 1996). The characterization of these remains using genetic markers offers a way to count and identify individuals in a population, determine their sex and movement patterns, infer parentage or relatedness, and assess pathogens and diet (Kohn and Wayne 1997).

For example, feces from coyotes (*Canis latrans*) collected in the Santa Monica Mountains National Recreational Area near Los Angeles were genotyped by means of DNA techniques (Kohn *et al.* 1999; Figure 4). The total population was estimated at about 38 individuals from the extrapolation of curves relating the total number of genotypes to the number of feces typed. This value is very close to that estimated by using traditional trapping methods. Movement patterns were also inferred, and home ranges suggested by the distribution of genotypes were similar to those found with radiotelemetry methods (Figure 4). In addition, pairwise relatedness values were calculated between genotypes, showing that the population contained a high proportion of close relatives, as might be

expected from its small size and geographic isolation. At least one parent–offspring pair was identified.

Non-invasive approaches are very promising methods for monitoring threatened populations, as they avoid the disruption and possible harm caused by handling. However, non-invasive methods will not entirely replace the need for ecological monitoring, and it is critical to understand the amount of effort involved and the importance of choosing the right models to analyze the data (Mills *et al.* 2000; Morin *et al.* 2001; Waits *et al.* 2001).

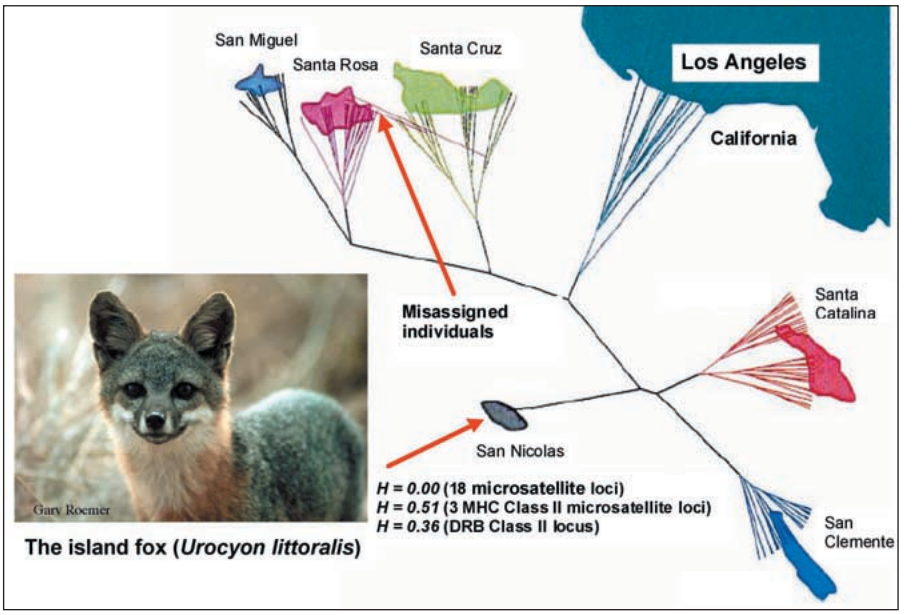

Figure 3. *Relationship tree of individual foxes inhabiting different Channel Islands, California. The tree shows that with the exception of two noted individuals, all foxes are most genetically similar to others from their population of origin (as indicated by different colored lines). San Nicolas Island foxes have no genetic variation and thus only one genotype line is observed (Gilbert* et al. *1990; Wayne* et al. *1991). However, for microsatellite loci associated with the MHC, about half the individuals are heterozygous and about 36% are heterozygous for DNA sequences from the DRB Class II gene (Aguilar* et al. *in press).*

Historic and ancient DNA

The vast majority of population genetic studies use a current sample of individuals to reconstruct past events and historic patterns of variation. However, this requires assumptions to be made about the continuity of populations and the processes which generate divergence between them. A direct historical perspective can be obtained from preserved remains, ranging from the vast holdings of museums to the remains preserved in natural deposits. For example, historic museum specimens less than 200 years old were used to address the origins and relationships of the red wolf (*Canis rufus*) and the Hawaiian Laysan duck (*Anas laysanensis*), and the loss of genetic variation in the northern elephant seal (*Mirounga angustirostris*), the northern hairy-nosed wombat (*Lasiorhinus krefftii*), the greater prairie chicken (*Tympanuchus cupido*), the Chinook salmon (*Oncorhynchus tshawytscha*), and the endangered Hawaiian goose (*Branta sandvicensis*)(Wayne *et al.* 1999; Hofreiter *et al.* 2001).

A longer perspective is provided by ancient material preserved in caves (Hadly *et al.* 1998) and Arctic permafrost for as long as 50 000 years. For example, DNA studies of contemporary North America brown bears (*Ursus arctos*) have shown that current patterns of genetic subdivision do not reflect a long history of isolation (Waits *et al.* 1998; Leonard *et al.* 2000; Barnes *et al.* 2002; Figure 5). The DNA showed that bears having sequences from the four clades that are geographically distinct today were previously sympatric, as all four clades were found inhabiting the Fairbanks-Dawson area of Alaska 37 000–42 000 years BP (Leonard *et al.* 2000; Figure 5). Subsequently, these sequences became partitioned geographically by a process of colonization and founding events. Moreover, three distinct and previously unrecognized periods of population turnover were uncovered (Barnes *et al.* 2002). The first period was defined by sequences from specimens older than 35 000 years BP, the second by sequences from specimens 21 000–10 000 years BP, and finally by sequences from modern populations (Barnes *et al.* 2002).

This succession is best interpreted as reflecting extinction followed by replacement with a large founding population, and is probably due to habitat or biotic changes. Understanding how environmental changes affected large mammal demography in the past is critical to understanding how future changes will affect the ecosystems on which similar animals now depend. Recently, DNA analysis of soil samples from Arctic permafrost cores identified plant and animal taxa that were present, suggesting that whole plant and animal communities can be reconstructed (Willerslev *et al.* 2003).

Adaptation and evolutionary history

The vast majority of conservation genetic evaluations are based on neutral markers which are influenced by genetic drift and, with one exception, are the most appropriate for addressing the questions discussed above and in Table 1. Specifically, neutral markers may often be poor surrogates for levels of variation in fitness traits (Reed and Frankham 2001). Furthermore, measures of population differentiation based on the analysis of quantitative traits, such as life history characteristics, may not be well correlated with measures based on neutral markers (McKay and Latta 2002; Merila and Crnokrak 2001). Conservation units based on historical isolation alone

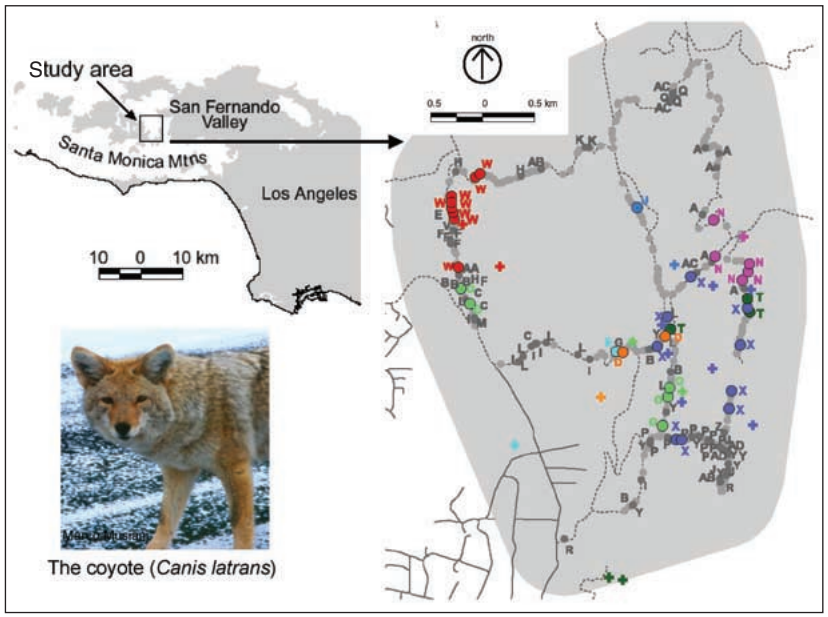

Figure 4. *Study area in the Santa Monica Mountains near Los Angeles, California, where non-invasive fecal survey of coyotes was undertaken (Kohn* et al. *1999). The location of feces collected (all dots) and typed feces (dark grey and colored dots) are shown in the map on the right. The multilocus genotype of each typed feces is designated as a letter, and colored dots are those genotypes common to a local area. Radiotelemetry studies showed that this was the focal area of the individuals with lettered genotypes (Kohn* et al. *1999).*

may not capture the adaptive variation necessary for populations to thrive in the short and long term, given changing environmental conditions (Crandall *et al.* 2000). Consequently, conservation genetic surveys should include neutral markers to assess population history and demography, as well as assays of fitness-related traits to preserve adaptive diversity. Fitness-related markers alone are not sufficient as conservation tools, because natural selection will bias inferences about population history and demography.

The importance of measuring variation in fitness-related traits is exemplified by the San Nicolas Island fox subspecies. Previous genetic surveys have shown that the island population is the most monomorphic sexually reproducing animal population yet described (Wayne *et al.* 1991; Goldstein *et al.* 1999). Such monomorphism implies lower fitness and an inability to respond to changing environmental conditions. However, this diagnosis was based on loci not influenced by selection. A group of occasionally surveyed functional loci in vertebrates are located in the major histocompatibility complex (MHC), and influence parasite resistance, mate recognition, and maternal–fetal interaction (Potts and Wakeland 1990; Edwards and Hedrick 2000). A recent survey of the San Nicolas Island foxes found high levels of variation in four MHC linked loci, suggesting intense balancing selection (Aguilar *et al.* in press; Figure 3). In this example, a demographic simulation showed that only a severe population bottleneck of ten individuals or less, during which intense selection occurred, could account for the high variation in the MHC and the absence of variation in neutral hypervariable markers (Aguilar *et al.* in press). This demonstrates the ability of selection to rescue variation in fitness-related genes that may be missed in conventional conservation genetics surveys of neutral loci.

The list of functional candidate genes is currently restricted to those derived from genome sequencing efforts in model organisms, limiting the range of taxa that can be surveyed. However, in the future, new high-throughput molecular approaches may be used to directly identify genes that are the object of natural selection in specific populations (Kohn *et al.* 2000; Schlotterer 2003). Even today, a range of molecular approaches may allow the assessment of adaptive variation in candidate genes (Ford 2000; van Tienderen *et al.* 2002; Purugganan and Gibson 2003; Luikart *et al.* 2003). Nevertheless, a widely accepted approach for quantifying adaptive variation at the molecular level remains an elusive goal.

Given the current lack of a comprehensive assay for fitness-related genes, conservation assessments especially need to consider adaptive aspects of phenotypes, and the environment in which populations live. For example, if the polar bear (*Ursus maritimus*) is placed in the brown bear phylogeny in Figure 5, they group with brown bears of Alaska's ABC Islands (clade II). A strict application of the genealogical ESU concept (Moritz 1994) would not place them in a separate ESU, because polar bear sequences do not define a polar bear-only group (ie a monophyletic group). In contrast, the phenotype distinction of the polar bear is profound, including a suite of adaptations for life in the high Arctic. In addition, the polar bear lives predominantly in the tundra, a habitat environmentally distinct from the boreal forest home of the brown bear, and no one would realistically include the polar bear in the same ESU as ABC Island brown bears.

However, this cursory comparison suggests a more general methodology for assessing both the evolutionary and adaptive divergence of populations (Crandall *et al.* 2000). The former is revealed through the historical perspective provided by molecular genetic analysis, whereas the latter is provided by an analysis of natural history, functional aspects of the genotype and phenotype, and habitat data. In fact, the "ESU-or-not" distinctions are a bit contrived, and species should generally be managed as a network of populations connected by various degrees of gene flow and migration (Crandall *et al.* 2000). For long-isolated populations, gene flow should not be encouraged, whereas the conservation goal for other populations should be to restore historic levels of gene flow. By considering genetic,

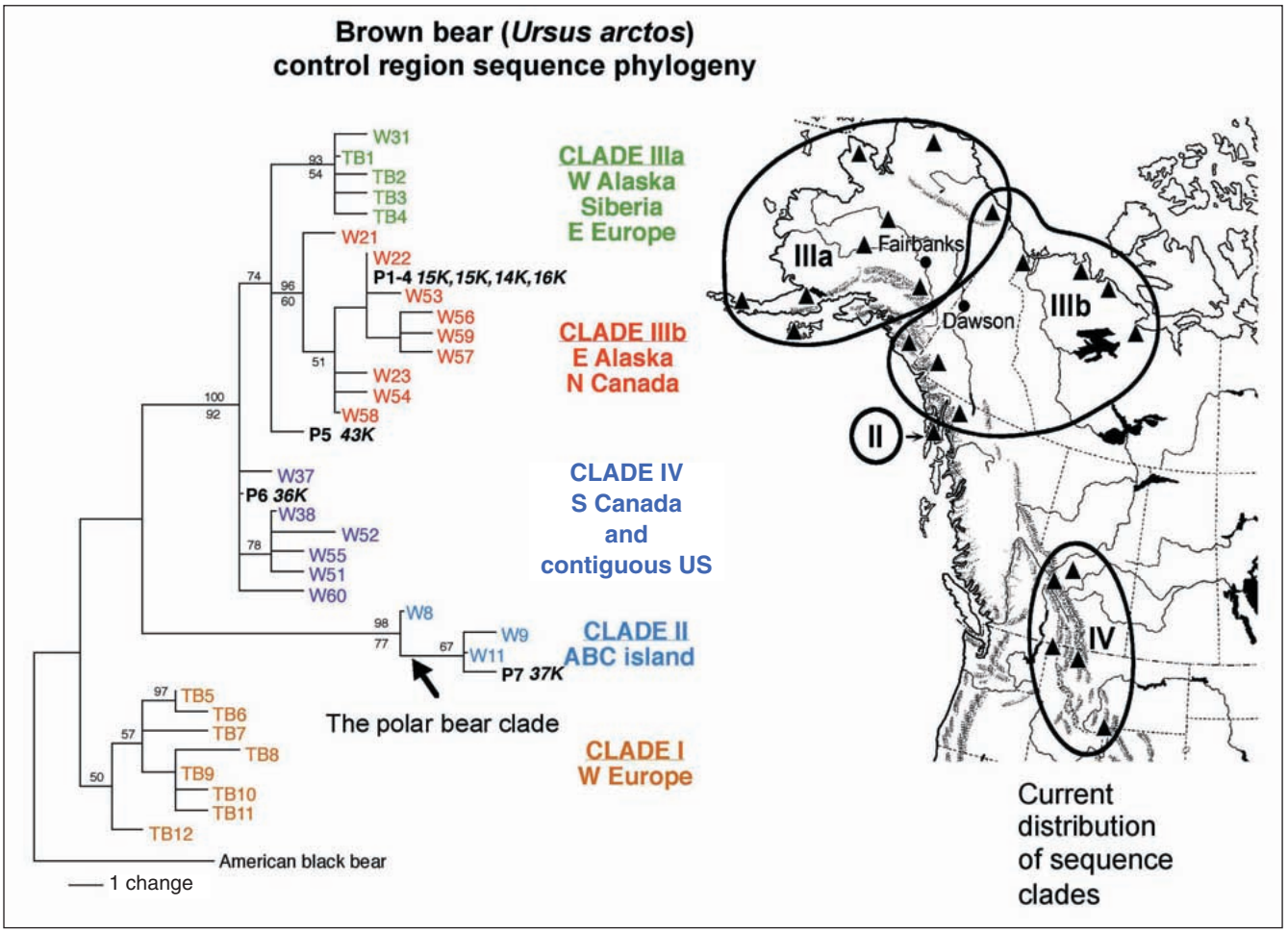

Figure 5. *North American and European brown bear sequence clades as defined by phylogenetic analysis of mitochondrial DNA control region sequences (left) from Leonard* et al. *(2000). Clade IV sequences are found in US and southern Canadian bears ("grizzly bears"). Clade IIIa sequences are found in Western Alaska and Europe, whereas clade IIIb sequences are found in eastern Alaska and Northern Canada. Clade II sequences are found in bears from the ABC islands off the coast of British Columbia, a clade that also includes sequences from polar bears. The geographic distribution of clades (right) shows largely distinct boundaries. Sequences P1–P7 (black) are ancient sequences from brown bear remains in the Arctic permafrost with radiometric dates as shown. These ancient sequences show that the current geographic pattern (right) was not in existence 36 000–43 000 years ago, when sequences related to all four clades were found in the Fairbanks-Dawson area.*

phenotypic, and ecological data, we may obtain the information most relevant to the survival of populations, and gain a forum for joint genetic and ecological studies.

Challenges

A primary goal of conservation biology is to enhance the long-term survival of species and the ecosystems on which they depend. We must therefore expand the focus of molecular studies to include the use of markers that more directly assay traits relevant to individual survival and reproduction. This requires jointly planned genetic and ecological studies, aimed at understanding variation in genes affecting fitness and how adaptive variation changes with environments. Rather than dominating the field of conservation genetics, molecular techniques should be complemented by studies of behavior and ecology.

The power of these techniques will probably increase dramatically in the near future, as high-throughput sequencing and genotyping become more widely used and genome sequencing projects of model species provide functional genes that can be surveyed. These techniques will enable a new series of questions to be addressed, which should focus on issues relevant to population persistence and restoration.

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